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(57) Abstract

Polypeptides that bind CD4, including the HIV-1 coat proteins gp120 and gp41, reduce migration of $CD4^+$ cells such as monocytes and T cells in response to chemoattractants, including C-C chemokines, SDF-1α, and fMLP. Such polypeptides are useful for treating inflammatory conditions and diseases.

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THERAPEUTIC CHEMOKINE ANTAGONISTS

CROSS-REFERENCE TO RELATED CASES

This application claims the benefit of U.S. Provisional Application No. 60/024,708, filed September 6, 1996, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates to a new class of C-C chemokine antagonists that are useful for treating inflammatory conditions or diseases, for example.

Chemokines are peptides that mediate chemotaxis and other pro-inflammatory phenomena (Schall, *Cytokine* 3:165, 1991). The family of chemokines is subdivided into two distinct subfamilies, C-X-C and C-C, based on the arrangement of the first two cysteines of the primary amino acid sequence.

Members of the C-C chemokine subfamily have remarkable similarities in their structural organization and biochemical properties and have similar biological effects, both *in vitro* and *in vivo*. For example, RANTES, MIP-1α, and MIP-1β are C-C chemokines that are all potent inducers of T-cell and mononuclear phagocyte chemotaxis and exert diverse effects on eosinophilic and basophilic polymorphonuclear leukocytes (Schall, *Cytokine* 3:165, 1991). The biological properties of C-C chemokines have prompted speculation that these chemokines are mediators in autoimmune and allergic disorders.

Chemokines bind to a variety of receptors that contain seven-transmembrane regions and thus are known as STM receptors. In the nanomolar (nM) range, chemokines activate G proteins, initiate signal transduction, and mobilize receptor-expressing inflammatory cells. Higher (μM) doses bind to STM receptors but uncouple G proteins, resulting in an attenuation or desensitization of leukocyte response and a concomitant inhibition of migration. Several receptors that bind RANTES, MIP-1α, and MIP-1β with high affinity have recently been identified and cloned (Neote *et al.*, *Cell* 72:415, 1993; Gao *et al.*, *J. Exp. Med.* 177:1421, 1993; and Combadiere *et al.*, *J. Mol. Biol.* 60:147-152, 1996). RANTES, MIP-1α, MIP-1ß, and MCP-1 genes have been isolated and expressed in heterologous systems (see, e.g., Schall and Bacon, *Curr. Opin. Immunol.* 6:865-873, 1994.

The human immunodeficiency virus (HIV) utilizes chemokine receptors, such as CCR5 and fusin, as co-receptors to enter monocytes and T lymphocytes (Berger *et al.*, *Science* 272:872-877, 1996; Jon, *Science* 272:809-810, 1996). HIV-1 infection can be blocked by MIP-1-α, MIP-1-β, and RANTES (Cocchi *et al.*, *Science* 270:1811-1815, 1995).

SUMMARY OF THE INVENTION

It has been discovered that the HIV-1 proteins gp120 and gp41 interfere with the capacity of various chemokines to bind to their receptor(s) on $CD4^+$ cells such as monocytes and T-cells, for example, and "desensitize" the chemotactic response of the cells to chemokines by down-regulating chemokine receptor expressi n. More generally, CD4-binding substances, of which gp120 and gp41

are representative, suppress $CD4^+$ cells such as monocytes and T cells to chemoattractants such as chemokines, reducing migration of such cells to an inflammatory site at which such chemoattractants are produced. As a result, such substances can be used to treat various acute and chronic inflammatory conditions or diseases, for example.

According to one embodiment of the invention, pharmaceutical compositions are provided for reducing chemotaxis of a $CD4^+$ cell in response to a chemoattractant such as a C-C chemokine (e.g., MIP-1α, MIP-1β, RANTES, MCP-1, and MCP-3), SDF-1α, and fMLP. Such pharmaceutical compositions comprise an effective amount of one or more of gp120, gp41, and variants thereof, and a pharmaceutically suitable excipient. Variants of gp120 and gp41 useful in the practice of the present invention include, but are not limited to, the V3 loop or PND domain of gp120 and CD4-binding fragments of gp120 and gp41 such as peptide F. Such pharmaceutical compositions can also include an anti-inflammatory substance other than gp120, gp41, or variants thereof.

According to another embodiment of the invention, methods are provided for reducing a chemotactic response of a $CD4^+$ cell that comprise administering a pharmaceutical composition that includes an effective amount of one or more of gp120, gp41, or variant forms thereof. According to a related embodiment of the invention, methods are provided for treating inflammatory conditions or diseases comprising administering to a mammal (e.g., a human) a pharmaceutical composition that includes an amount of one or more of gp120, gp41, and variant forms thereof, that is effective in significantly reducing a chemotactic response of a monocyte or T lymphocyte and a pharmaceutically suitable excipient.

According to another embodiment of the invention, methods of treating an inflammatory condition of a subject (e.g., a human subject) are provided that comprise administering to the subject a pharmaceutical composition comprising an amount of a CD4-binding compound that is effective to significantly reduce migration of said $CD4^+$ cells to an inflammatory site.

According to another embodiment of the invention, methods are provided for inhibiting chemotaxis of a $CD4^+$ cell comprising contacting the cell with an effective concentration of a CD4-binding compound.

According to another embodiment of the invention, assays for chemokine activity or chemokine binding are provided wherein a $CD4^+$ cell is contacted with a test substance and the effect of the test substance on migration of the cell is determined.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows that five different preparations of purified gp120, from various, laboratory-adapted strains of HIV-1, display dose-dependent chemotaxis effects on fresh, human peripheral

blood monocytes in the dose range of 0.1 to 50 nM, similar to the effects of chemokines MCP-1, MIP-1α and MIP-1β.

FIG. 2 shows that preincubation with gp120 (E109) inhibited the binding of I^{125}-radiolabeled MIP-1β to the surface of monocytes. The degree of competitive binding was roughly equivalent to that shown by MIP-1ß. The inhibition of binding of I^{125}-radiolabeled MIP-1β was significantly increased by preincubation of monocytes with gp120.

FIG. 3 shows inhibition of binding to monocytes of I^{125}-radiolabeled MIP-1β, MIP-1α, and RANTES, mediated by gp41-2, a gp41 preparation.

FIGS. 4A-E show reduction of binding of ^{125}I-labeled C-C chemokines to monocytes pretreated with gp120 (shaded bars), and competition by gp120 for chemokine binding to monocytes (hatched bars). Horizontal lines depict the maximal level of direct competition of chemokine binding by unlabeled native ligands (60-120 nM). A, MIP-1β; B, MIP-1α; C, RANTES; D, MCP-1; E, MCP-2.

FIG. 4F shows the effect of gp120 on neutrophil binding of ^{125}I-IL-8. Shaded bars represent the percent reduction of neutrophil binding of labeled IL-8. Hatched bars indicate the levels of direct competition with IL-8 for binding by gp120. The horizontal line depicts the maximal level of direct competition of IL-8 binding by unlabeled IL-8 (60 nM).

FIG. 5 shows inhibition of monocyte binding for MIP-β by anti-CD4 monoclonal antibodies. *$P<0.05$ in comparison to MIP-1β binding to cells incubated with medium.

FIGS. 6A-E shows the percent inhibition by gp41 of total binding of various C-C chemokines by monocytes. Hatched bars show results of direct competition of gp41 with C-C chemokines for monocyte binding. Shaded bars show results of preincubation of monocytes with gp41. A, MIP-1β (120 nM); B, MIP-1α (120 nM); C, RANTES (120 nM); D, MCP-1 (120 nM); E, MCP-3 (120 nM).

FIG. 6F shows the percent inhibition of total binding of the C-X-C chemokine IL-8 by neutrophils after treatment with 0-5 nM gp41. Hatched bars show results of direct competition of gp41 with IL-8 for monocyte binding. Shaded bars show results of preincubation of monocytes with gp41.

FIG. 7 shows fluorescence-activated cell sorting (FACS) analyses showing cell surface expression of CCR5 by monocytes after preincubation with gp41. A, untreated cells; B, MIP 1-β; C, gp41; D, anti-CD4 monoclonal antibody; E, PMA; F, anti-CD14 monoclonal antibody.

FIG. 8 shows the inhibitory effect of gp120 (MN) on binding of SDF-1α by T cells after pretreatment with herbimycin A.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Unless otherwise indicated below, terms used herein are to be understood according to their rdinary significance as used by those of ordinary skill in the art. See, e.g., *Dorland's Illustrated Medical Dictionary*, Philadelphia: W.B. Saunders Company, 1985 (26th ed.) for definitions of medical terms.

"Variants" f gp120 and gp41. A "variant" according to the present invention is a molecule that is derived from gp120 or gp41 and that is active in an assay as described herein. One example of a variant of gp120 or gp41 is a fragment thereof that retains gp120 or gp41 biological activity, e.g., that inhibits a C-C cytokine from binding monocytes and/or that antagonizes chemotaxis, preferably a CD-4 binding fragment such as peptide F (EGSDTITLPCRIKQFINMWQE), a fragment of gp120 or the following peptide fragments derived from the DP178 region of gp41 that potently inhibit HIV-1 virus entry and replication:

LAI YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF

MN YTSLIYSLLEKSQTQQEKNEQELLELDKWASLWNWF

RF YTGIIYNLLEESQNQQEKNEQELLELDKWASLWNWF

SF2 YTNTIYTLLEESQNQQEKNEQELLELDKWASLWNWF

Fragments of gp120 or gp41 are preferably at least 10-50, more preferably 10-20 amino acids in length.

"Variant" polypeptides also include amino-acid sequence variants of a native or wild-type gp120 or gp41 polypeptide or fragment thereof that include substitutions, deletions, or insertions of one or a small number of amino acid residues but retain the ability to inhibit a C-C cytokine from binding monocytes or that antagonizes chemotaxis. Such amino-acid sequence variants have at least 70% amino-acid sequence homology with the corresponding wild-type polypeptide, preferably at least 80%, more preferably at least 90%, and most preferably at least 95% homology. Exemplary of such conservative replacements are: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; valine to isoleucine or leucine.

Other "variant" polypeptides include conventional protein modifications to increase stability or solubility or to alter protein configuration, including the incorporation of a rare amino acid, a D-amino acid, a glycosylation site, or a cysteine for disulfide-bridge formation.

A variant can be synthesized chemically, or the isolated gene can be site-directed mutagenized, or a synthetic gene can be synthesized and expressed in bacteria, yeast, baculovirus, tissue culture, etc., by conventional methods. The genetic construct can be designed to produce a gene fusion or to add C-terminal or N-terminal amino acid residues that would facilitate purification by trapping on columns or use of antibodies. Conventional methods for producing variant polypeptides are describe, for example, in *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 (hereinafter, "Sambrook *et al.*, 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al.*, Greene Publishing and Wiley-Interscience, New Y rk, 1992 (with periodic updates) (hereinafter, "Ausubel *et al.*, 1992). Techniques for chemical synthesis of polypeptides are described, for

example, in Merrifield, *J. Amer. Chem. Soc.* 85:2149-2156, 1963.

The gp120 protein has five loops. The V3 loop stimulates production of neutralizing antibodies that bind to HIV-1, preventing it from infecting cells (Brolinden *et al.*, *Proc. Nat'l Acad. Sci.* 89:461, 1992). While V3 is a variable region, the immunodominant neutralizing domain, PND, differs only slightly among isolates. Thus, antibodies to PND neutralize large numbers of HIV-1 strains (Berman *et al.*, *J. Virol.* 66:4464, 1992).

In one set of experiments, two V3-loop fragment preparations, V3BP and V3MNP, were observed to inhibit binding of RANTES, MIP-1α, and MIP-1ß. V3BP and V3MNP are described by Berzofsky *et al.*, *Annals of N.Y. Acad. of Sci.* 754: 161-8 (1995). Accordingly, polypeptides that include a V3 loop of gp120 are encompassed by the variant gp120 polypeptides of the present invention. The amino acid sequence of the V3 loop and residues associated with known immunological responses to V3 (neutralization epitopes, antibody response in humans, and T-cell response) are provided, for example, in Moore and Nara, *AIDS 1991 5* (suppl. 2):S21-S33 (see, especially FIG. 2). Purified V3 and fragments of V3 are commercially available, for example, from Intracell (Cambridge, MA).

Drug discovery; Assays for chemokine binding and chemotaxis. According to another embodiment of the invention, assays are provided for identifying inhibitory factors useful as antiinflammatory agents, i.e., substances that bind to CD4 and have anti-chemotactic effects. Binding assays and assays related to chemotaxis and calcium mobilization are described in the Examples below. Other binding assays and assays related to determination of chemotaxis and calcium mobilization are discussed, for example, in Xu *et al.*, *Eur. J. Immunol.* 25:2612-2617, 1995. Conventional *in vivo* assays chemotaxis, e.g., conventional assays for leukocyte infiltration into skin or peritoneal fluid of mice following injection of chemokines, can be used to confirm the antiinflammatory activity of a test substance (see, e.g., Wang *et al.*, *Methods: A Companion to Methods in Enzymology* 10:135-144, 1996).

Therapeutic uses and pharmaceutical compositions. The proteins gp120 and gp41, and variants thereof interfere with migration of inflammatory cells *in vivo* and therefore are useful as anti-inflammatory agents. These polypeptides are useful, for example, in treating a wide range of autoimmune, allergic, and other acute and chronic inflammatory diseases and conditions in which chemotaxis of $CD4^+$ cells (e.g., monocytes, eosinophils and T cells) in response to a chemoattractant (e.g., a chemokine) results in migration of the cells to a site of inflammation. Such inflammatory and autoimmune diseases and conditions include, but are not limited to: multiple sclerosis; rheumatoid arthritis; glomerulonephritis; post-viral myocarditis; atherosclerosis, hypersensitivity reactions (types I-IV, e.g., contact dermatitis, eczema, acute and chronic allergic reactions such as asthma, and autoimmune disease), etc. Other inflammatory or autoimmune diseases or conditions that can be treated using the pharmaceutical compositions of the present inventi n are described, for example, in Harrison, *Principles of Internal Medicine* (McGraw Hill 1994). Pharmaceutical compositions according to the present invention can also be used for immunosuppression, e.g., in the

context of organ transplantation. Embodiments of the present invention are useful therapeutically, i.e., curing or reducing the severity of a disease or condition, and prophylactically, i.e., reducing or avoiding one or more symptoms of a disease or condition.

Pharmaceutical formulations. Pharmaceutical compositions according to the present invention encompass formulations comprising (1) an amount (for example, a unit dosage) of one or more polypeptides according to the present invention together with (2) one or more well-known non-toxic pharmaceutically acceptable excipients, including carriers, diluents, and/or adjuvants, and optionally (3) one or more biologically active ingredients other than gp120, gp41, or a variant thereof. Standard pharmaceutical formulation techniques are used, such as those disclosed in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA (latest edition).

The pharmaceutical composition can be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions (*e.g.*, eye or ear drops, throat or nasal sprays, *etc.*), transdermal patches, and other forms known in the art.

Such pharmaceutical compositions can be administered systemically or locally in any manner appropriate to the treatment of a given condition, including orally, parenterally, rectally, nasally, buccally, vaginally, topically, optically, by inhalation spray, or via an implanted reservoir. The term "parenterally" as used herein includes, but is not limited to subcutaneous, intravenous, intramuscular, intrasternal, intrasynovial, intrathecal, intrahepatic, intralesional, and intracranial administration, for example, by injection or infusion. Parenteral administration, e.g., intravenous, intramuscular, etc., is a preferred mode of administration for many inflammatory conditions, since gp120, gp41, and variants thereof distribute well throughout the body.

Pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins (such as human serum albumin), buffers (such as phosphates), glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wool fat, for example.

Tablets and capsules for oral administration can be in a form suitable for unit dose presentation and can contain conventional pharmaceutically acceptable excipients. Examples of these include binding agents such as syrup, acacia, gelatin, sorbitol, tragacanth, and polyvinylpyrrolidone; fillers such as lactose, sugar, corn starch, calcium phosphate, sorbitol, or glycine; tableting lubricants, such as magnesium stearate, talc, polyethylene glycol, or silica; disintegrants, such as potato starch; and dispersing r wetting agents, such as sodium lauryl sulfate. The tablets can be coated according to methods well kn wn in normal pharmaceutical practice. Oral liquid preparati ns can be in the form of, for example, aqueous r ily suspensions, solutions, emulsi ns, syrups r

elixirs, or can be presented as a dry product for reconstitution with water or ther suitable vehicle before use. Such liquid preparations can contain conventional additives such as suspending agents, *e.g.*, sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats, emulsifying agents, *e.g.*, lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (including edible oils), *e.g.*, almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives such as methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavoring or coloring agents.

Pharmaceutical compositions according to the present invention can also be administered parenterally in a sterile aqueous or oleaginous medium. The composition can be dissolved or suspended in a non-toxic parenterally-acceptable diluent or solvent, e.g., as a solution in 1,3-butanediol. Adjuvants such as local anesthetics, preservatives, and buffering agents can also be dissolved in the vehicle. Commonly used vehicles and solvents include water, physiological saline, Hank's solution, Ringer's solution, and sterile, fixed oils, including synthetic mono- or di-glycerides, etc. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as Ph. Helv or a similar alcohol.

For topical application, the drug may be made up into a solution, suspension, cream, lotion, ointment in a suitable aqueous or non-aqueous vehicle. Additives may also be included, e.g., buffers such as sodium metabisulphite or disodium edeate; preservatives such as bactericidal and fungicidal agents, including phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents, such as hypromellose.

The dosage unit involved depends, for example, on the condition treated, nature of the formulation, nature of the condition, embodiment of the claimed pharmaceutical compositions, mode of administration, and condition and weight of the patient.

Further information for formulating pharmaceutical compositions according to the present invention can be found, for example, in Remington, *Pharmaceutical Sciences*, Easton, PA: Mack Publishing Co.; *The National Formulary*, Washington: American Pharmaceutical Association; Goodman and Gilman's, *The Pharmacological Basis of Therapeutics*, Gilman *et al.* (eds.), New York: Macmillan; and Munson, *Principles of Pharmacology*, New York: Chapman and Hall.

Active ingredients that can be included in pharmaceutical compositions according to the present invention include, but are not limited to, one or more well-known antiinflammatory, immunomodulatory, or immunosuppressive substances such as cyclophosphamides, methotrexate, cyclophosphamide, antimalarials, glucocorticoids (e.g., hydrocortisone), cyclosporine A, azathioprine, penicillamine, antilymphocyte globulin (ALG), FK506, rapamycin, gold salts, interferons, etc.

"Effective amount". By "effective amount" is meant the quantity of a c mposition acc rding to the invention that is sufficient to reduce the chemotactic response of a target cell, e.g., a monocyte or T cell, to a chemokine by a statistically significant degree. Preferably, the amount

of the composition is effective to prevent, to cure, or at least reduce one or more symptoms of an inflammatory response, condition or disease, whether acute or chronic. Amounts effective for this use depend on the severity of the disease and the weight and general state of the patient.

Dosages used *in vitro* provide useful guidance in the amounts useful for *in vivo* administration of the pharmaceutical composition, and animal models may be used to determine effective dosages for treatment of particular disorders. See, e.g., Goodman and Gilman, *supra*, and Remington, *supra*. For example, an effective *in vivo* concentration of peptide F is expected to be in the range of about 0.25 μg/mL to about 2500 μg/mL, preferably about 2.5 μg/mL to about 250 μg/mL. Similarly, an effective *in vivo* concentration of gp120 is expected to be in the range of about 0.05 μg/mL to about 500 μg/mL, preferably about 0.5 μg/mL to about 50 μg/mL. (Conversion to mg/kg body weight can be approximated by the conversion factor 1000 mL ≈ 1 kg body weight of subject). The administered dosage would be calculated to achieve such an effective concentration in blood serum or another body fluid or in a tissue, e.g., at the affected site. The dosage can be adjusted by determining the concentration of peptide F or gp120, for example, in blood serum (or another body fluid or tissue) of a subject by conventional methods.

The compositions and methods of the present invention are useful for treatment of humans or for veterinary purposes, i.e., for treatment of animals, e.g., non-human mammals.

The invention will be better understood by reference to the following Examples, which are intended to merely illustrate the best mode now known for practicing the invention. The scope of the invention is not to be considered limited thereto, however.

EXAMPLES

EXAMPLE 1: HIV Envelope gp120 Activates CD4 to Desensitize Chemoattractant Receptors on Monocytes

HIV-1 infection is initiated by high-affinity binding of the virus envelope glycoprotein, gp120, to CD4, the primary receptor for HIV-1 (Klatzmann *et al.*, *Nature* 312:767-768, 1984; Dalgleish *et al.*, *Nature* 312:763-767, 1984). Members of the STM chemokine receptor superfamily have been identified as co-receptors for HIV-1 infection (Bleul *et al.*, *Nature* 382:829-832, 1996; Oberlin *et al.*, *Nature* 382:833-835, 1996; Feng *et al.*, *Science* 272:872-877, 1996; Deng *et al.*, *Nature* 381:661-666, 1996; Dragic *et al.*, *Nature* 381:667-673, 1996; Alkhatib *et al.*, *Science* 272:1955-1958, 1996; Liu *et al.*, *Cell* 86:367-377, 1996). HIV-1 cell-type tropism seems to be dictated by chemokine receptor usage and T-cell line tropic viruses appear to use CXCR4, while monocytotropic viruses primarily use CCR5 as fusion co-receptors. A minority of HIV-1 strains may also use other C-C chemokine receptors such as CCR2b and CCR3 as their fusion cofactors (Oberlin *et al.*, *Nature* 382:833-835, 1996; Feng *et al.*, *Science* 272:872-877, 1996; Deng *et al.*, *Nature* 381:661-666, 1996; Dragic *et al.*, *Nature* 381:667-673, 1996; Alkhatib *et al.*, *Science* 272:1955-1958, 1996; Liu *et al.*, *Cell* 86:367-377, 1996). Dual tropic HIV-1 strains presumably interact with either type of chemokine receptors. Furthermore, both T lymphocytes and m nocytes express CXCR4 as well as the C-C receptors (Loetscher *et al.*, *J. Biol. Chem.* 269:232-237, 1994).

Nevertheless, purified gp120 from monocytotropic virus has been shown to competitively inhibit MIP-1β binding to CCR5 present on T lymphocytes, and this binding competition was greatly enhanced by gp120-CD4 interactions (Trkola *et al.*, *Nature* 384:184-187, 1996; Wu *et al.*, *Nature* 384:179-183, 1996). In contrast, gp120 from T-cell-line adapted viruses did not compete with MIP-1β for binding to CCR-5, in agreement with infection tropism studies.

Although HIV-1-induced T lymphocyte suppression and depletion is a central feature of the immunosuppression, monocyte-macrophages are also targets of the virus infection. Earlier studies demonstrated that monocytes from AIDS patients exhibited a significantly compromised capacity to migrate in response to several chemotactic factors, including activated complement component C5a, bacterial peptide fMLP, and undefined lymphocyte-derived chemotactic factors (Smith *et al.*, *J. Clin. Invest.* 74:2121-2128, 1984). Incubation of monocytes with soluble gp120 has also been reported to suppress the expression of receptors for C5a and fMLP (Wahl *et al.*, *J. Immunol.* 142:3553-3559, 1989). We therefore sought to test whether gp120, which is shed in copious quantities in AIDS patients (Gelderblom *et al.*, *Virology* 156:171-176, 1987) may contribute to the anti-inflammatory and immunosuppressive effects of HIV-1 by antagonizing interactions of chemoattractant receptors on monocytes with their ligands.

Natural gp120 was isolated from the culture fluid of HIV-1 (MN or RF)-infected H9 cells by immunoaffinity chromatography (Pyle *et al.*, *J. Virol.* 62:2258-2264, 1988). Recombinant gp120 (strain MN and IIIB) and recombinant soluble CD4 were purchased from Intracel (Cambridge, MA). At the highest concentration tested, the gp120 preparations had <0.2 ng/mL of endotoxin activity. Monoclonal and anti-CD4 antibodies were purchased from Biogenesis (Poole, United Kingdom). Both clone A6 and E9 were able to stain CD4 on the cell surface and clone E9 was able to block HIV binding, as described by the manufacturer. Monoclonal and anti-CD14 antibody (clone K4) was purchased from DAKO Corp. (Carpinteria, CA). Recombinant chemokines were from PeproTech (Rocky Hill, NJ). (Recombinant chemokines are also available, for example, from Sigma Immunochemicals [St. Louis, MO] and R&D Systems [Minneapolis, MN]). Radioiodinated chemokines were purchased from Dupont NEN (Boston, MA). Human peripheral blood monocytes were isolated from Buffy Coat (NIH Clinical Center, Transfusion Medicine Department, Bethesda, MD) enriched for mononuclear cells by using isosmotic Percoll gradient as described (Xu *et al.*, *Eur. J. Immunol.* 25:2612-2617, 1995). Neutrophils were isolated from Buffy Coat blood with Dextran sedimentation as described (Xu *et al.*, *Eur. J. Immunol.* 25:2612-2617, 1995). The purity of the cell preparations was examined by morphology and was $>90\%$ for monocytes and $>98\%$ for neutrophils. The CCR3 and CCR5 transfected HEK 293 cells were obtained from Dr. P. Gray, ICOS Corp., Seattle, WA). CCR1 and CXCR4/fusin cDNA were isolated in this laboratory and were transfected into 293 cells as described (Ben-Baruch *et al.*, *J. Biol. Chem.* 270:22123-22128, 1995). CCR2b cDNA was supplied by Dr. I. Charo (University of California at San Francisco, San Francisco, CA) and was stably transfected into 293 cells in this laboratory. The viability of monocytes or HEK293 cells before and after gp120 treatment was examined by trypan-blue exclusion

and was more than 95% after up to 18 hr treatment at 37°C.

Binding assays were performed by preincubating duplicate samples of monocytes (2 x 10^6/sample) or chemokine receptor transfected 293 cells (1 x 10^6/sample) with different concentrations of gp120 for 60 min at 37°C in a volume of 200 μL/sample of binding medium (RPMI 1640, 1% bovine serum albumin, 5 mM HEPES, and 0.5% sodium azide). ^{125}I-labeled chemokines (0.12 nM) were then added to each sample. To parallel duplicate samples, different concentrations of gp120 or unlabeled chemokines (as controls) were added simultaneously with radiolabeled chemokines. After incubation at room temperature for 40 min, the cells were centrifuged through a 10% sucrose/phosphate buffered saline (PBS) cushion and the cell-associated radioactivity was measured in a gamma counter (Clinigamma-Pharmacia, Gaithersburg, MD). The percentage reduction of chemokine binding to monocytes by gp120 treatment was calculated by the formula: (1 cpm associated with cells preincubated with gp120)/(cpm associated with cells incubated with medium alone) x 100.

To determine the change in the number of binding sites and affinity for a given chemokine, cells were preincubated with or without gp120 (25 nM) for 60 min at 37°C. The duplicate monocyte samples were then incubated with 0.12 nM radiolabeled chemokines in the presence of increasing concentrations of unlabeled chemokines. The cells were pelleted after exposure for 40 min at room temperature and measured for radioactivity. The binding data were analyzed with the Macintosh computer program LIGAND (Dr. P. Muson, DCRT, National Institutes of Health, Bethesda, MD).

Chemotaxis assays were performed using a 48-well chemotaxis chamber (Neuroprobe, Cabin John, MD) as described (Xu *et al.*, *Eur. J. Immunol.* 25:2612-2617, 1995). 25-μL aliquots of chemokines diluted in chemotaxis medium (RPMI 1640 containing 10 mg/ml bovine serum albumin, 25 mM Hepes) were placed in the lower wells of the chamber (Neuroprobe, Cabin John, MD). Monocytes or neutrophils (50 μL cell suspension in the same medium at 2 x 10^6/mL) or HEK 293 cells (1 x 10^6/mL) were placed in the upper wells. The upper and lower wells were separated by a polycarbonate filter (5 μm pore size, polyvinylpyrrolidone-free for neutrophils; Nucleopore, Pleasanton, CA). For 293 cells the filter of 10 μm pore size was precoated with collagen IV as described (Ben-Baruch *et al.*, *J. Biol. Chem.* 270:22123-22128, 1995). After incubation (90 min for monocytes, 60 min for neutrophils, 5 hr for 293 cells) in air with 5% CO_2 at 37°C, the filters were removed, fixed, and stained with Diff-Quik (Harleco, Gibbstown, NJ), and the number of cells that migrated across the filters were counted after coding the samples. Results were calculated as the mean (± SD) number of migrated cells in three high-powered light microscopy fields (400 x) in triplicate samples. The chemotaxis index (C.I.) was calculated as follows: C.I. = (Number of migrated cells in response to chemoattractant)/(Number of randomly migrated cells [in response to medium]). In chemotaxis deactivation experiments, monocytes (or neutrophils) and HEK 293 cells transfected with chemokine recept rs were preincubated with chemoattractants or gp120 for 30 min r 60 min at 37°C then washed 3x with PBS. The cell migration in response to various chemoattractants was assessed and computed as described above. After subtraction f background

migration (in response to medium), the percentage deactivation of chemotaxis to a given chemoattractant was calculated by the formula: [1 - (Migration of cells preincubated with gp120)/(Migration of cells preincubated with medium)] x 100.

Internalization of CXCR4/fusin was examined by pretreatment of $CD4^+/CXCR4^+$ HEK 293 cells with gp120 and anti-CD4 antibody. HEK293 cells expressing CXCR4 and intact CD4 were pretreated for 3 hr at 37°C with recombinant gp120 (MN, 25 nM) or anti-CD4 monoclonal antibody (A6, 5 μg/mL). The cells were centrifuged onto slides and permeabilized. The slides were then stained with an anti-CXCR4/fusin monoclonal antibody (12G5) followed by incubation with FITC-labeled goat anti-mouse IgG $F(ab')_2$ fragments. Slides were examined using a Zeiss 310 Confocal Laser Scanning Microscope (Carl Zeiss). Nomarski, FITC (488 nm, green) and DAPI (UV 364 nm, blue) images were prepared for each specimen and colored images were superimposed on Nomarski.

All experiments were performed at least three times and the results presented are either from a representative experiment or from a pool of experiments. The significance of the difference between experimental and control groups was analyzed with the Student T test.

In initial experiments, five different preparations of purified gp120 from various laboratory-adapted strains of HIV-1 were found to display dose-dependent chemotaxis effects on fresh, human peripheral blood monocytes in tests conducted with the cytokines MCP-1, MIP-1α and MIP-1β (FIG. 1). It was also demonstrated that radiolabeled MIP-1ß is competitively inhibited from association to monocytes in a dose-dependent manner by addition of gp120. When a gp120/E109 preparation and MIP-1β are added at the same time, binding of MIP-1β to monocytes was competitively inhibited (FIG. 2). However, the inhibitory effect was much greater when the monocytes were preincubated at 37°C with gp120 for 60 to 90 minutes before the addition of MIP-1β. The inhibitory effect of the gp120 was comparable in potency to that of the ligand, MIP-1β itself. Since MIP-1β is known to use only the CCR5 receptor, the gp120 also interacted with the CCR5 receptor. Since preincubation increased the inhibitory effect, and, in addition, since gp120 was active at relatively low concentrations, these results showed that gp120 also has desensitizing effects on the receptor(s), in addition to competitive effects. Similarly, gp41 was demonstrated to inhibit binding of RANTES, MIP-1α and MIP-1β in a dose-dependent fashion (FIG. 3).

The effect of gp120 on chemokine binding was further investigated by adding soluble gp120 purified from HIV (MN) (Pyle *et al.*, *J. Virol.* 62:2258-2264, 1988) and radiolabeled chemokines simultaneously to human peripheral blood monocytes as described (Xu *et al.*, *Eur. J. Immunol.* 25:2612-2617, 1995). Monocytes were preincubated in duplicate with medium alone or with different concentrations of purified gp120 (MN) for 60 min at 37°C. ^{125}I-labeled chemokines were added and the cultures were incubated for 40 min at room temperature. The cells were then centrifuged through a sucrose cushion and measured for radioactivity. Direct competition experiments were performed in parallel by adding different concentrations of gp120 simultaneously with ^{125}I-labeled chemokine to duplicate monocyte samples. Unlabeled chemokines were used as control competitors. The cells were incubated at room temperature for 40 min and harvested. FIG.

4 shows one of three representative experiments. While all unlabeled chemokines (60-120 nM) showed significant competition for binding by radiolabeled ligands (FIG. 4, horizontal lines), gp120 resulted in only a weak or no competition for binding sites with radiolabeled chemokines on monocytes or neutrophils (FIG. 4, hatched bars). This is in agreement with the report that gp120 from T cell-line adapted subtype B strains such as MN and IIIB failed to compete for MIP-1β binding to T cell CCR5 following brief pre-incubation at 4°C (Trkola *et al.*, *Nature* 384:184-187, 1996).

In contrast to the weak or no competition observed with gp120 chemokine, preincubation of monocytes with soluble gp120 of the MN strain at 37°C for 60 min resulted in a marked dose-dependent inhibition of the capacity of monocytes to bind C-C chemokines (FIGS. 4A-E, shaded bars). The IC50 for gp120 inhibition of monocyte binding for chemokines (0.1, 1.0, and 5.0 nM for MIP-1α, MIP-1β, and RANTES, respectively) was similar to that for the native ligands when they were used as direct competitors (0.3, 0.6, and 1.0 nM for MIP-1α, MIP-1β, and RANTES, respectively). Consequently, preincubation of monocytes with gp120 not only markedly inhibited their capacity to bind MIP-1β, which utilizes exclusively CCR5 (Murphy, *Cytokine and Growth Factor Reviews* 7:47-64, 1996; Combadiere *et al.*, *J. Leuk. Biol.* 60:147-152, 1996), but also inhibited their binding of MIP-1α and RANTES, which in addition use CCR1 (Combadiere *et al.*, *J. Leuk. Biol.* 60:147-152, 1996; Neote *et al.*, *Cell* 72:415-425, 1993) and other C-C chemokine receptors (Premak and Schall, *Nature Med.* 2:1174-1178, 1996; Murphy, *Cytokine and Growth Factor Reviews* 7:47-64, 1996). Furthermore, the capacity of monocytes to bind ^{125}I-MCP-1 and ^{125}I-MCP-2 was inhibited up to 50% by preincubation of the cells with gp120 (FIGS. 4D, 4E), even though these chemokines have not been reported to inhibit HIV replication and predominantly use CCR2b (Premak and Schall, *Nature Med.* 2:1174-1178, 1996; Xu *et al.*, *Eur. J. Immunol.* 25:2612-2617, 1995) and CCR1 (Gong et al., unpublished), respectively. Preincubation of monocytes with native and recombinant gp120 from T cell-line adapted virus as well as recombinant gp120 from a monocytotropic strain, CM, all interfered with the capacity of monocytes to bind chemokines. Taken together, these results show that the observed reduction of chemokine binding to monocytes preincubated with soluble gp120 is more promiscuous than the reported capacity of chemokines to competitively inhibit HIV-1 entry and envelope-mediated cell fusion (Cocchi *et al.*, *Science* 270:1811-1815, 1995; Cocchi *et al.*, *Nature Med.* 2:1244-1247, 1996).

The C-X-C chemokine IL-8 is chemotactic for neutrophils rather than monocytes. Preincubation of neutrophils, which do not express CD4, with gp120 did not result in any reduction of IL-8 binding to neutrophils (FIG. 4F). Since gp120 inhibits the capacity of CD4$^+$ monocytes, but not CD4$^-$ neutrophils, to bind chemokines, the effect of gp120 was tested on CD4$^-$ HEK 293 cells transfected to express only one of the chemokine receptors. HEK 293 cells transfected to express CCR5, CCR1, or CXCR4 showed specific binding and chemotaxis in response to their specific ligands, but preincubation of these cells with gp120 from either MN or IIIB strains did not affect chemokine binding. This suggested that the inhibition by gp120 of the capacity of chemokine receptors to bind their ligands may require additional cellular molecules such as CD4.

Interaction of CD4 with gp120 had been reported to transduce signals resulting in chemotactic migration and Ca^{++} mobilization in $CD4^+$ cells, including monocytes and T lymphocytes (Kornfeld *et al.*, *Nature* 335:445-448, 1988; Cruikshank *et al.*, *Biomed. Pharm.* 44:5-11, 1990). We therefore examined the possibility that CD4 may mediate the inhibition by gp120 of monocyte binding for chemokines. The inhibitory effect of preincubation with gp120 (MN strain) on monocyte binding of C-C chemokines was blocked by pretreatment of gp120 with soluble CD4. This suggests that soluble CD4 may sequester gp120 and prevent its binding to cell membrane anchored CD4 and the subsequent chemokine receptor down-regulation (Table 1). To determine the effect of anti-CD4 antibodies on monocyte binding of chemokines, monocytes were preincubated with recombinant gp120 (rgp120) (MN and IIIB) or different concentrations of monoclonal anti-CD4 antibodies for 60 min. at 37°C). ^{125}I-MIP-1 was added to the cells at room temperature for 40 min. The cells were then pelleted through a sucrose cushion and cell-associated radioactivity was determined. Antibodies from clones A6 and E9 stained $CD4^+$ cells, but only clone E9 blocked HIV binding by HIV-1 (as per Biogenics Poole, UK). Unlabeled MIP-1β was used to define the level of maximal direct competition by native ligand. These experiments demonstrated that preincubation with monoclonal anti-CD4 antibodies emulated the effect of gp120 in inhibiting monocyte binding for MIP-1β, whereas anti-CD14 monoclonal antibody had no effect (FIG. 5). Although only the E9 anti-CD4 blocks binding of HIV-1, the A6 antibody, which does not block HIV-1, was more potent in inhibiting the capacity of monocytes to bind chemokines.

In additional experiments, $CD4^+$/$CXCR4^+$ HEK293 cells were pretreated with gp120 and anti-CD4 antibody. Confocal microscopy of FITC-stained cells showed that CXCR4/fusin localized to the cell surface in medium-treated (control) cells but internalized into the perinuclear cytoplasm in response to the native ligand of CXCR4, SDF-1α (1 μg/mL) in both $CD4^-$ and $CD4^+$ cells. Preincubation with gp120 (IIIB, 25 nM) and anti-CD4 (5 μg/mL) resulted in a marked internalization of CXCR4/fusin in cells co-expressing CD4 but not in $CD4^-$ cells. The apparent requirement for initial interaction of gp120 with CD4 is in agreement with the recent report that preincubation with gp120 of $CD4^+$/fusin $(CXCR4)^+$ T cells resulted in the formation of a gp120/CD4/fusin complex that could be co-precipitated with antibodies against any one of these three molecules (Lapham *et al.*, *Science* 274:602-605, 1996). However, our results also indicate that the interaction between CD4 and CD4-binding substances such as gp120 results in internalization of chemokine receptors and inhibits the ability of $CD4^+$ cells to bind chemokines.

The HIV-1 gp120 and CD4/co-receptor-mediated cell fusion was reported to be resistant to pertussis toxin, suggesting that the virus competes for chemokine receptor occupancy without activating G-protein signaling (Cocchi *et al.*, *Nature Med.* 2:1244-1247, 1996; Oravecz *et al.*, *J. Immunol.* 157:1329-1332, 1996). However, the need to preincubate monocytes for 1 h with gp120 in our study suggested that binding of CD4 by gp120 may result in signal transduction. This conclusion is supported by reports that gp120 is chemotactic for monocytes (Kornfeld *et al.*, *Nature* 335:445-448, 1988; Cruikshank *et al.*, *Biomed. Pharm.* 44:5-11, 1990). We observed that both

native and recombinant preparations of MN and native lymphocytotropic RF strain of gp120 induced monocyte migration. The chemotactic effect of gp120 purified from a native MN strain was inhibited by neutralizing anti-gp120 and was also sensitive to pertussis toxin at concentrations that did not affect spontaneous cell migration, suggesting that gp120 can activate G-proteins.

Since preincubation with gp120 from T cell-line adapted (MN, IIIB) and monocytotropic viruses (CM) could inhibit the capacity of monocytes to bind chemokines, we determined whether gp120 could also inhibit monocyte chemotaxis in response to chemokines. In fact, preincubation of monocytes with gp120 for 60 min at 37°C markedly reduced the chemotactic response of these cells to C-C chemokines as well as to SDF-1α, the ligand for CXCR4/fusin (Table 2). This effect is characteristic of "heterologous" desensitization by a chemoattractant resulting in the down-regulation of the receptors for unrelated chemoattractants that use similar G protein-coupled seven transmembrane (STM) receptors (Wang *et al.*, *J. Exp. Med.* 177:669-703, 1993). Preincubation of monocytes with gp120 MN for a short period (up to 30 min at 37°C) had no effect, whereas chemokine ligands resulted in the expected "homologous" desensitization of chemotaxis. The heterologous desensitization of monocyte migration by gp120 could also be demonstrated for fMLP (Table 2), which utilizes an STM receptor with some homology to the chemokine receptors. This observation confirmed an earlier report that long term preincubation of monocytes with gp120 significantly down-regulated cell surface expression of receptors for fMLP as well as C5a (Wahl *et al.*, *J. Immunol.* 142:3553-3559, 1989). However, gp120 failed to desensitize directional migration of chemokine receptor-transfected HEK 293 cells lacking CD4 in response to their respective chemokines. In addition, as shown in Table 2, preincubation of gp120 with soluble CD4 completely abolished the inhibitor effect of gp120 on monocyte migration induced by chemokines and fMLP, further supporting a critical role of cell membrane CD4 molecules in mediating the action of gp120. This inhibitory effect of gp120 (MN) on monocyte migration was also totally eliminate by preincubation with a monoclonal anti-gp120 (MN) antibody. As expected from the inability of gp120 to down-regulate chemokine binding sites on neutrophils, gp120 did not desensitize the chemotactic response of neutrophils to fMLP or IL-8.

The binding of gp120 with CD4 may compete with chemokines simply by causing conformation changes, resulting in the exposure of an epitope(s) that enables the gp120 to occupy chemokine receptors (Trkola *et al.*, *Nature* 384:184-187, 1996; Wu *et al.*, *Nature* 384:179-183, 1996). Preincubation with gp120 may additionally trigger intracellular signals, such as PKC or arachidonic acid activation (Parada *et al.*, *Cell Immunol.* 168:100-106, 1996; Wahl *et al.*, *Proc. Natl. Acad. Sci. USA* 86:621-625, 1989), that desensitize and inactivate chemoattractant receptors. Furthermore, PMA, a PKC activator, down-regulated HIV-1 fusion co-factor, later identified as CXCR4/fusin (Lapham *et al.*, *Science* 274:602-605, 1996) and inhibited T cell viral entry (Golding *et al.*, *J. Virol.* 68:1962-1969, 1994). This latter pathway is supported by data showing that addition of a low dose of the protein kinase inhibitor staurosporine was able to reverse the down-regulation by gp120 of MIP-1β binding to monocytes (Table 4). The ability of monocytes to bind other C-C

chemokines and SDF1α could also be protected by pretreatment with staurosporine before the cells were exposed to gp120. A more specific PKC inhibitor, calphostin C, could mimic the protective effect of staurosporine. These observations suggest that binding of gp120 to CD4 on monocytes activates intracellular protein kinase activity, which in turn down-regulates chemoattractant receptor expression and function.

The possibility that CD4 signals are required to down-regulate the chemoattractant receptors was further supported by experiments showing that the ability of HEK 293 cells to bind MIP-1β could not be inhibited by preincubation of the cells with gp120 or anti-CD4 antibody if the cells were expressing CCR5 and CD4 lacking intracytoplasmic tail, whereas the binding capacity of the cells transfected with intact CD4 were inhibited by gp120 or anti-CD4 antibody (Table 3), even though cells containing tailless or intact CD4 were both >90% positive by anti-CD4 staining and fluorescence-activated cell sorting (FACS) analysis. The effect of gp120 on cells expressing CCR5 and intact CD4 could be eliminated by pretreatment of gp120 with soluble CD4. These observations again suggest that CD4 is an active rather than a passive participant in the down-regulation of chemokine receptor expression by gp120.

The idea that gp120 heterologously desensitizes chemoattractant receptors on monocytes was supported by Ca^{++}-mobilization experiments. Analysis of the changes in intracellular Ca^{++} mobilization was performed suing a spectrofluorometer (Delta-scan, Photon Technologies International, Princeton, NJ) as described (McKeating and Willey, *AIDS 1989*, 3 (suppl. 1):S35-S41; Freed and Risser, *Bull. Inst. Pasteur* 88:73-110, 1990). Briefly, monocytic cells were incubated at a concentration of 10^7/mL for 60 min at 37°C in RPMI 1640 containing 10% FCS and Indo-1AM (2 μM; Molecular Probes, Eugene, OR). After washing once with medium, the cells were suspended in DPBS with Ca^{++}, Mg^{++}, and 5 mM glucose. Indo-1 excitation was assessed at 358 nm with detection of bound dye at 402 nm (violet) and free dye at 486 (blue). Gp120 (MN) at 50 nM did not induce significant Ca^{++} mobilization in monocytes under our experimental conditions, nor did it attenuate the monocyte response to a subsequent challenge (100 sec after gp120) by chemokines or fMLP. However, preincubation of monocytes with gp120 (MN, 50 nM) for 1 h at 37°C almost completely abolished the Ca^{++} mobilization in these cells induced by MIP-1α, RANTES, SDF1α (12 nM), as well as fMLP (10 nM). The Ca^{++} mobilization induced by MCP-1 (12 nM) was also significantly reduced by preincubation of monocytes with gp120 (50 nM) (Ueda *et al.*, unpublished).

In our experiments, natural and recombinant gp120 from T cell-tropic strains MN, IIIB, and RF, as well as recombinant monocytotropic CM, all exhibited similar inhibitory effects on the capacity of monocytes to bind C-C chemokines and desensitized the monocyte chemotactic responses to C-C chemokine and fMLP. This is consistent with observations that the CD4 binding region of gp120 is conserved and consequently that gp120 from primary isolates (Volsky *et al.*, *J. Virol.* 6:3823-3833, 1996) and T cell-line adapted viruses all can down-regulate chemoattractant recept r expression on monocytes.

A 20 amino-acid sequence of gp120 from the IIIB strain, peptide F

(EGSDTITLPCRIKQFINMWQE) has been found to be a potent chemoattractant f r monocytes, and its activity was abolished by soluble CD4 (Ueda and Wang, unpublished).

In this study, we report for the first time that down-regulation by gp120 of C-C chemokine receptors requires signal transduction by intact CD4 molecules on the cell surface. Although gp120 had the most potent effects on monocyte interaction with MIP-1α, MIP-1β, and RANTES, which are CCR5 ligands, significant inhibition was also observed with other C-C chemokines, as well as with SDF-1α and fMLP, chemoattractants that use receptors other than CCR5, suggesting that gp120 causes a heterologous desensitization of monocyte responses to chemoattractants. All preparation of gp120 from T cell-tropic and monocytotropic sources inhibited monocyte migration in response to chemokines and fMLP.

Down-regulation of chemokine receptors by gp120 may interfere with the initial entry of HIV-1 into CD4 cells, provided sufficient soluble "shed" gp120 is available to internalize all the receptors. Although in the course of the disease some of the gp120 detected in the sera of patients may in fact be complexed by anti-gp120, it is unlikely that the effect of gp120 is blocked by anti-gp120 antibodies, because there are considerable variations in the specificity, amount, and timing of anti-gp120 antibody production (Moore *et al.*, *J. Virol.* 68:5142-5155, 1994; Moore and Jarrett, *AIDS Res. Hum. Retroviruses* 4:369-379, 1988). In most patients, serum anti-gp120 antibodies appear relatively late and were first detected only after p24 antigen and infectious-virus titer in the peripheral blood had already declined many-fold from their highest values (Moore *et al.*, *J. Virol.* 68:5142-5155, 1994). Many of the heterologous anti-gp120 antibodies do not actually have the capacity to recognize the CD4 binding conformation on gp120 as indicated by the failure of these antibodies to compete for gp120 binding to CD4 with a neutralizing anti-gp120 human monoclonal antibody that does recognize the conformational CD4-binding structure on gp120 (Ho *et al.*, *J. Virol.* 65:489-493, 1991). Such neutralizing antibody appeared later in the patients' sera than other anti-gp120 antibodies that do not block CD4-binding structure on gp120 (Ho *et al.*, *J. Virol.* 65:489-493, 1991). Since CD4-blocking anti-gp120 antibodies appear relatively late after infection, there is ample opportunity for the virus-associated or "shed" gp120 (Gelderblom *et al.*, *Virology* 156:171-176, 1987) to interact with CD4 on immune cells. Furthermore, the affinity of binding of gp120 to CD4 is high, comparable and competitive with antibody binding affinities (Nara *et al.*, *FASEB J.* 5:2437-2455, 1991). In addition, gp120 that is not associated with virion can be detected on the surface of explanted CD4 T cells from patients (Sunila *et al.*, *AIDS* 11:27-32, 1997). In tissues such as lymphatic organs, the dense packing of cells could facilitate high affinity gp120-CD4 interaction even in the presence of excess circulating blocking antibodies (Mittler and Hoffmann, *Science* 245:1380-1382, 1989; Cruikshank *et al.*, *Biomed. Pharmacother.* 44:5-11, 1990), which may not block the binding of gp120 to CD4. These observations strongly argue that shed gp120 can have biological effects n circulating cells of infected individuals.

HIV-1 may be using envelope proteins such as gp120 to "disarm" the inflammat ry host defense processes. Such a mechanism would have profound implications for the pathogenesis of

HIV-induced disease. Our observati ns also provide a mechanistic basis for the phenomenon of viral interference (Volsky *et al.*, *J. Virol.* 6:3823-3833, 1996; Arya and Gallo, *Proc. Natl. Acad. Sci. USA* 93:4486-4491, 1996), since interaction of gp120 with CD4 can potentially make cells refractory to subsequent invasion by additional viruses by a general down-regulation of co-receptors.

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Table 1. Effect of preincubation with recombinant gp120 on the expression of chemokine binding sites by monocytes[a]

	Cells preincubated with					
	Medium		rgp120 (MN, 10 nM)		rgp120 (MN) & sCD4[b]	
^{125}I-chemokine	Kd(nM)	Sites/cell	Kd(nM)	Sites/cell (% reduction)	Kd(nM)	Sites/cell
MIP-1α	0.20	1500	0.30	600 (60)[c]	0.31	1200
MIP-1β	0.62	4500	1.33	1300 (71)[c]	0.84	3900
RANTES	0.35	4650	1.22	1100 (76)[c]	0.55	4050
MCP-1	2.10	6700	3.22	4500 (32)[c]	2.50	5900
MCP-2	1.80	8100	2.46	5300 (34)[c]	2.00	7500

[a] Monocytes were preincubated with medium alone or rgp120 (MN, 10nM) w/wo sCD4 for 60 min. at 37°C, and their capacity to bind C-C chemokines assessed. The binding data were analyzed using the Macintosh program LIGAND, and the results shown are representative of three experiments performed.

[b] rgp120 was preincubated with molar concentrations of sCD4 at a ratio of 1:25 for 30 min. and the mixture was then added to the monocyte cultures.

[c] $P < 0.05$ (Student t test) compared to cells incubated with medium alone.

Table 2. Effect of soluble CD4 (sCD4) on the inhibition by rgp120 (MN) of monocyte chemotaxis in response to C-C chemokines, SDF-1 and fMLP[a]

Chemoattractants	C. I. and (% Inhibition of Migration by preincubation with)		
	Medium	rgp120	rgp120 + sCD4
MIP-1	3.51	1.0 (100[b])	3.23 (11)
MIP-1	2.42	0.96 (100[b])	2.50 (0)
RANTES	3.13	1.0 (100[b])	2.70 (20)
MCP-1	4.60	3.0 (45[b])	3.92 (19)
MCP-2	3.62	2.57 (40[b])	3.62 (0)
FMLP	11.02	4.51 (65[b])	11.20 (0)
SDF-1	6.43	1.54 (90[b])	6.51 (0)

[a] Recombinant gp120 was preincubated in the presence or absence of sCD4 for 30 min. at 37°C, then was further incubated with monocytes for 60 min. at 37°C. After washing, the cell migration in response to C-C chemokines (10nM) SDF-1α (100 nM) or fMLP (10nM) was determined. The results represent a summary of five experiments performed.

[b] $P < 0.05$ (Student t test) compared to the migration of monocytes preincubated with medium alone.

Table 3. **Preincubation with gp-120 of CCR5 HEK 293 cells expressing intact CD4 down-regulates the cell binding for ^{125}I-MIP-1β[a]**

Cell Type	cpm (% Inhibition of Binding)			
	Preincubation of Cells With			
	Medium (binding in the absence of cold MIP-1β)	Medium[b] (binding in the presence of cold MIP-1β)	gp120 (MN, 25 nM)	Anti-CD4 (A6)
CCR5/293	4435	1101 (75)[c]	4306 (3)	4634 (0)
Control Vaccinia Vector	5927	1372 (74)[c]	5671 (4)	5452 (8)
VCB2 (Tailless CD4)	4203	1449 (66)[c]	3882 (8)	3981 (5)
VCB7 (Intact CD4)	4217	1027 (76)[c]	1796 (57)[c]	1991 (53)[c]

[a] CCR5/293 cells were infected with control vaccinia virus, or recombinant vaccinia virus encoding tailless CD4 (VCB2) or intact CD4 (VCB7) at 37°C for 1 h, at multiplicity of infection of 5 in DMEM containing 2% FCS and antibiotics. The cells were washed and were cultured for 10 h at 37°C in DMEM containing 400 μg/ml, 10% FCS and antibiotics. The cells were then gently detached with trypsin/EDTA, resuspended in binding medium, aliquoted, and incubated for 10 h in the presence or absence of gp120 or anti-CD4 mAb. Binding assays utilizing ^{125}I-MIP-1β were performed at room temperature for 40 min. Both VCB2 and VCB7 infected CCR5/293 cells were more than 90% CD4$^+$ as assessed by FACS analysis (not shown).

[b] Cells were preincubated with medium and bindings were performed in the presence of 120 nM unlabeled MIP-1β.

[c] Significant inhibition of ^{125}I-MIP-1β binding compared to cells preincubated with medium alone ($P < 0.05$). Preincubation of gp120 with soluble CD4 reversed the inhibitory effect of gp120 on cell binding for MIP-1β.

Table 4. Staurosporine reverses the inhibitory effect of gp120 on monocyte expression of MIP-1β binding sites[a]

Cell treatment					
Medium		rgp120 (MN, 25 nM)		Staurosporine followed by gp120[b]	
Sites/cell	Kd (nM)	Sites/cell	Kd (nM)	Sites/cell	Kd (nM)
1520	0.2	750[c]	0.3	1560	0.4
1460	0.3	780[c]	0.4	1370	0.5
1500	0.2	690[c]	0.5	1450	0.3
2000	0.4	990[c]	0.4	1950	0.5
3500	0.5	1350[c]	0.6	3180	0.7
2430	0.5	1100[c]	0.6	1240	0.5

[a] Monocytes were incubated with medium alone or recombinant gp120 (MN, 25 nM) for 60 min at 37°C. After washing, duplicate aliquots of the cells were incubated with 0.12 nM radioiodinated MIP-1β in the presence of increasing concentrations of unlabeled ligand. The samples were cultured at room temperature for 40 min then were harvested and measured for radioactivity. The data was analyzed with LIGAND program. Results obtained with cells from 6 donors are shown.

[b] Cells were first preincubated with Staurosporine (1 ng/ml) for 15 min at 37°C followed by treatment with gp120.

[c] Significant reduction of MIP-1β binding sites on monocytes treated with gp120 ($p < 0.05$, Student's t test).

EXAMPLE 2: Effect of gp41 on Monocyte Binding and Chemotactic Response to Chemokines

HIV-1 envelope protein gp41 plays a critical role in viral fusion and infection. After binding of gp120 to CD4 and subsequently to chemokine receptors, HIV-1 uses gp41 to fuse with and penetrate the target cell membrane, which leads to the injection of the viral genome into the target cell. In order to clarify whether HIV-1 may use gp41 in addition to gp120 to subvert the host immune response, we examined the affect of gp41 on monocyte binding and chemotactic response to chemokines.

Gp41 did not directly compete with C-C chemokines for binding sites on monocytes (FIGS. 6A-E, hatched bars). However, monocytes preincubated with gp41 for 60 min at 37°C showed remarkably reduced binding for a number of C-C chemokines, including MIP-1α, MIP-1β, RANTES, MCP-1, and MCP-3 (FIGS. 6A-E, shaded bars). The ability of neutrophils to bind the C-X-C chemokine IL-8 was not inhibited by preincubation of the cells with gp41 (FIG. 6F).

Fluorescence-activated cell sorting (FACS) analyses performed on human monocytes showed that the expression of CCR5 on monocytes was down-regulated by preincubation of the cells with gp41 (FIG. 7A, C). The degree of CCR5 down-regulation by gp41 was comparable to down-regulation by the CCR5 ligand MIP-1β and an anti-CD4 monoclonal antibody (FIG. 7B, D), whereas an anti-CD14 monoclonal antibody did not show any effect (FIG. 7F). PMA, a potent protein kinase C activator, also down-regulated cell surface expression of CCR5 (FIG. 7E).

The effect of soluble CD4 (sCD4) on the inhibition by gp 41 (MN) of monocyte chemotaxis in response to chemokines and fMLP was tested. Recombinant gp41 (0.5 nM) was preincubated in the presence or absence of sCD4 (10 nM) for 30 min at 37°C then further incubated with monocytes for 60 min at 37°C. After washing, the cell migration in response to chemokines (10 nM) or fMLP (10 nM) was determined. Gp41 was not chemotactic for monocytes and did not induce Ca^{++} flux in monocytes. However, after incubation with gp41, monocytes showed remarkably reduced chemotactic response (Table 5) and Ca^{++} flux induced by C-C chemokines and bacterial peptide fMLP. The inhibition of monocyte chemotactic response by gp41 to chemoattractants apparently required the presence of cellular CD4, because pretreatment of gp41 with a soluble CD4 reduced the effect of gp41 on monocytes (Table 5). In addition, gp41 did not inhibit the chemotactic response of CD4$^-$ neutrophils to the C-X-C chemokine IL-8 and fMLP. Furthermore, confocal microscopy showed that gp41 induced internalization of CXCR4/fusin in HEK 293 cells that co-express CD4 but not in HEK cells expressing only CXCR4/fusin.

We next investigated whether the inhibitory effect of gp41 on monocyte binding and chemotaxis to chemoattractants required activation of a signaling pathway leading to the down-regulation of chemoattractant receptors. CCR5/293 cells were infected with control vaccinia virus or recombinant vaccinia virus encoding tail-less CD4 (VCB2) or intact CD4 (VCB7) at 37°C for 1 hr at a multiplicity of infection (MOI) of 5 in DMEM containing 400 μg/mL, 10% FCS and antibi tics. The cells were then gently detached with trypsin/EDTA, resuspended in binding medium, aliquoted, and incubated for 10 hr in the presence or absence of gp41 or anti-CD4 monoclonal

antibody. Binding assays utilizing ^{125}I-MIP-1β were performed at room temperature for 40 min. Both VCB2 and VCB7 infected CCR5/293 were more than 90% CD4$^+$ as assessed by FACS analysis. The cells were preincubated with medium and binding was performed in the presence of 120 nM unlabeled MIP-1β. The requirement of signaling mediated by CD4 was suggested by the observation that the binding capacity for MIP-1β of HEK 293 cells was inhibited by gp41 only when the cells expressed both CCR5 and a CD4 molecule with the cytoplasmic domain but not a tail-less CD4 molecule, that is, CD4 lacking the cytoplasmic domain (Table 6).

We also examined the effect of gp41 on migration of CCR5/293/CD4 cells in response to MIP-1β. CCR5/293 cells were transfected with CD4 and were preincubated for 10 hr with gp41 (MN) or anti-CD4 antibodies at 37°C. The migration of HEK 293 cells induced by MIP-1β was also inhibited by gp41 when the cells expressed both CCR5 and an intact CD4 (Table 7).

In addition, we tested the effect of PKC inhibitors staurosporine or calphostin C on the inhibitory effect of gp 41. Monocytes were incubated with medium alone or recombinant gp41 (MN, 5 nM) for 60 min at 37°C. After washing, duplicate aliquots of the cells were incubated with 0.12 nM radioiodinated MIP-1β in the presence of increasing concentrations of unlabeled ligand. The samples were cultured at room temperature for 40 min, then harvested and measured for radioactivity. The data was analyzed with the LIGAND program. To test the effect of the PKC inhibitors, prior to treatment with gp41, cells were incubated with staurosporine (10 ng/mL) for 30 min or calphostin C (100 ng/mL) for 4 hr at 37°C. Pretreatment with staurosporine or calphostin C significantly reduced the inhibitory effect of gp41 on monocyte binding of the C-C chemokines MIP-1β and MCP-1 and the C-X-C chemokine SDF-1α (Table 8). These results suggest that gp41 inhibits monocyte binding and response to chemoattractants through a CD4-mediated signal transduction pathway in which PKC activation plays an important role.

Our data demonstrate for the first time that gp41 is a potent inhibitor of monocyte binding and function in response to chemoattractants. The effect of gp41 on monocytes requires the presence of CD4, which may activate protein kinases, resulting in the down-regulation of receptors for chemoattractants, including those chemokine receptors used by HIV-1 as fusion cofactors. Thus, by down-regulating chemokine receptors, gp41 may also contribute to the phenomenon of viral interference and provide a means by which HIV-1 disarms host defenses by inhibiting the capacity of monocytes to migrate to inflammatory sites.

EXAMPLE 3: Effect of gp120 on T Lymphocytes

The effect of gp120 (MN strain) on T lymphocytes was examined using the T lymphocyte leukemia cell line CEM (CD4$^+$) as well as peripheral blood T cells. CEM-SS cells (1 x 10^6) in 0.5% FBS-RPMI were incubated with herbimycin A (0, 0.1, 1.5, or 2.0 μM) for 18 hr at 37°C. After incubation with herbimycin A, the cells were incubated with gp120-MN (1 x 10^6 cells/tube/200 μL, 50 nM gp120-MN, 37°C for 60 min) and then tested for binding of SDF-1α. Gp120 did not directly compete with C-X-C chemokine SDF-1α for binding on CEM or blood CD4$^+$ T cells. After incubation of the cells with gp120, the binding of SDF-1α was significantly reduced (FIG. 8). This

down-regulation of SDF-1α binding n T cells by gp120 apparently requires signaling, since T cells pretreated with herbimycin A, a protein tyrosine kinase inhibitor, completely retained their capacity to bind SDF-1α (FIG. 8). These results suggest that gp120 was able to down-regulate CXCR4/fusin expression on T cells through a mechanism that involves the activation of tyrosine kinase(s). Thus, like its effect on monocytes, gp120 may also inhibit the T cell response to chemokines by down-regulating chemokine receptor expression on cell surfaces.

Table 5 Effect of soluble CD4 (sCD4) on the inhibition by gp41 (MN) of monocyte chemotaxis in response to chemokines and fMLP [a]

Cell	Chemoattractants	Monocytes chemotaxis (Chemotaxis index)		
		Medium	Cells preincubation with gp41 (% reduction)	gp41+ sCD4 (% recovery)
Monocytes	MIP-1β	2.73	0.99 (100)[b]	1.88 (51)[c]
	RANTES	2.35	1.06 (96)[b]	1.45 (30)[c]
	MCP-1	6.74	3.60 (55)[b]	6.85 (100)[c]
	SDF-1α	4.95	1.23 (94)[b]	3.70 (66)[c]
	fMLP	12.38	5.80 (58)[b]	8.35 (39)[c]
Neutrophils	IL-8	9.36	10.47 (0)	9.76
	fMLP	7.45	8.36 (0)	8.00

[a] Recombinant gp41 (0.5 nM) was preincubated in the presence or absence of sCD4 (10 nM) for 30 min. at 37°C, then was further incubated with monocytes for 60 min. at 37°C. After washing, the cell migration in response to chemokines (10 nM),or fMLP (10 nM) was determined.

[b] P<0.01 (Student t test) compared to the migration of monocytes preincubated with medium alone.

[c] P<0.01 (Student t test) compared to the migration of monocytes preincubated with gp41 alone.

Table 6 Preincubation with gp41 of CCR5 HEK293 cells expressing intact CD4 down-regulates the cell binding for ^{125}I-MIP-1β [a]

Cell Type	cpm (% Inhibition of Binding)			
	Preincubation of Cells with			
	Medium (binding in the absence of cold MIP-1β)	Medium [b] (binding in the presence of cold MIP-1β)	gp41 (MN, 25 nM)	Anti-CD4 (A6, 10 μg/ml)
CCR5/293	4435	1101 (75) [c]	4735 (0)	4634 (0)
Control Vaccinia Vector	5927	1371 (74) [c]	5467 (8)	5452 (8)
VCB2 (Tailless CD4)	4203	1449 (66) [c]	3745 (11)	3981 (5)
VCB7 (Intact CD4)	4217	1027 (76) [c]	1576 (62) [c]	1991 (53) [c]

[a] CCR5/293 cells were infected with control vaccinia virus, or recombinant vaccinia virus encoding tailless CD4 (VCB2) or intact CD4 (VCB7) at 37°C for 1h, at multiplicity of infection of 5 in DMEM containing 2% FCS and antibiotics. The cells were washed and were cultured for 10 h at 37°C in DMEM containing 400 μg/ml, 10% FCS and antibiotics. The cells were then gently detached with trypsin/EDTA, resuspended in binding medium, aliquoted, and incubated for 10 h in the presence or absence of gp41 or anti-CD4 mAb. Binding assays utilizing ^{125}I-MIP-1β were performed at room temperature for 40 min. Both VCB2 and VCB7 infected CCR5/293 were more than 90% CD4$^+$ as assessed by FACS analysis (not shown).

[b] Cells were preincubated with medium and binding were performed in the presence of 120 nM unlabeled MIP-1β.

[c] Significant inhibition of 125I-MIP-1β binding compared to cells preincubated with medium alone ($P < 0.05$).

Table 7 Effect of gp41 on CCR5/293/CD4 cell migration in response MIP-1β [a]

Chemoattractant	Medium	Chemotaxis Index (Mean +/- SE, % inhibition) Cellswere preincubated for 10 h. with		
		gp41 (MN, 25 nM)	Anti-CD4 (A6, 10 µg/ml)	Anti-CD4 (E9, 10 µg/ml)
Medium	1.0	1.0	1.0	1.0
MIP-1β (100 ng/ml)	15.3 +/- 1.5	6.3 +/- 2.0 (63)[b]	7.8 +/- 1.1 (52)[c]	6.9 +/- 0.8 (38)[c]

[a] CCR5/293 cells were transfected with CD4 and were preincubated for 10 h with gp41(MN) or anti-CD4 antibodies at 37°C. Cell migration in response to MIP-1β was then evaluated.

[b] $P<0.05$, [c] $P<0.01$ (Student t test) compared to the migration of monocytes preincubated with medium alone.

Table 8 Staurosporine and Calphostin C reverse the inhibitory effect of gp41 on monocyte expression of chemokine binding sites[a]

	Cell treatment							
	medium		gp41 (MN, 5 nM)		Staurosporine followed by gp41[b]		Calphostin C followed by gp41[c]	
Chemokine	Kd (nM)	Sites/cell	Kd (nM)	Sites/cell	Kd (nM)	Sites/cell	Kd (nM)	Sites/cell
MIP-1β	1.1	9600	1.2	2700[d]	0.9	4800[e]	---	---
MIP-1β	1.2	8100	1.1	4320[d]	---	---	1.9	6370[e]
SDF-1α	6.0	10607	6.0	4100[d]	4.0	8400[e]	---	---
SDF-1α	4.0	9960	6.0	3300[d]	---	---	4.0	4320[e]
MCP-1	1.1	9153	0.8	2228[d]	1.0	6684[e]	---	---
MCP-1	0.9	6243	0.7	1999[d]	---	---	1.0	4163[e]

[a] Monocytes were incubated with medium alone or recombinant gp41 (MN, 5 nM) for 60 min. at 37°C. After washing, duplicate aliquots of the cells were incubated with 0.12 nM radioiodinated MIP-1b in the presence of increasing concentrations of unlabeled ligand. The samples were cultured at room temperature for 40 min. then were harvested and measured for radioactivity. The data was analyzed with LIGAND program. Results are from representive experiments out of 12 performed.

[b] Cells were first preincubated with Staurosporine (10 ng/ml) for 30 min. at 37°C followed by treatment with gp41.

[c] Cells were first preincubated with Calphostin C (100 ng/ml) for 4 hours at 37°C under a light followed by treatment with gp41.

[d] $p < 0.05$ (Student t test) compared to cells treated with medium alone.

[e] $p < 0.05$ (Student t test) compared to cells treated with gp41 alone.

EXAMPLE 4: A gp120 Peptide that Includes the CD4 Binding Domain Inhibits Chemoattractant Receptors on Monocytes

In order to identify epitopes on gp120 that are critical for the suppression of monocyte receptors for chemoattractants, we used a synthetic 20 amino acid peptide corresponding to the CD4 binding region of gp120. This peptide (gp120 MN aa 414-434, designated peptide F) was found to be a potent chemoattractant for human monocytes and T lymphocytes. It is also moderately chemotactic for human neutrophils at high concentration. Preincubation of monocytes with peptide F for 1 h at 37°C significantly reduced cell binding of a great variety of chemokines, including MIP-1β, MCP-1, RANTES, and SDF-1α (Table 9). The monocyte migration in response to these chemokines as well as to bacterial fMLP was concomitantly reduced. The inhibitory effect of this peptide on monocyte response to chemoattractant was reversed by pretreatment of peptide F with soluble CD4 (Table 9), suggesting that peptide F utilizes CD4 to inhibit monocyte response. Thus, we have identified a CD4 binding domain of gp120 MN that mimics the inhibitory effect of full-length gp120 on chemoattractant receptors on monocytes.

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications that are within the spirit and scope of the appended claims.

TABLE 9 **Peptide F inhibits monocyte chemotaxis in response to chemokines and fMLP**

Preincubation : at 37°C for 60 min.

Chemoattractants	No. of Migrated Monocytes after Preincubation with			
	Medium	gp120 (414-434)	MIP-1β	FMLP
Medium	72 ± 13	70 ± 2	83 ± 8	47 ± 3
gp120 (414-434)	208 ± 16	111 ± 10** (70)	163 ± 15 (42)	45 ± 4*** (100)
MIP-1β	183 ± 22	67 ± 10** (100)	51 ± 5** (100)	45 ± 7** (100)
FMLP	947 ± 131	511 ± 61* (50)	490 ± 10* (54)	90 ± 10** (95)

Chemoattractants	No. of Migrated Monocytes after Preincubation with			
	Medium	gp120 (414-434)	RANTES	MCP-1
Medium	30 ± 1	31 ± 1	38 ± 3	27 ± 2
gp120 (414-434)	107 ± 5	71 ± 7* (48)	54 ± 7** (78)	55 ± 7** (64)
RANTES	69 ± 4	34 ± 2** (92)	32 ± 4** (100)	29 ± 1*** (95)
MCP-1	236 ± 24	172 ± 2* (32)	146 ± 13* (47)	40 ± 3*** (94)

Results are expressed as Chemotaxis Index ± standard error of migration.
The numbers in brackets are percent of inhibition.
Significant difference (*: P<0.05,**: P<0.01,***: P<0.001) between with and without chemoattractants by T-TEST analysis.

gp120 (414-434: INTRACELL): 1 μM, RANTES: 12nM. MCP-1, 12nm. MIB-1β; 12nM. FMLP, 10nM.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition for reducing chemotaxis of a $CD4^+$ cell to in response to a chemoattractant, the composition comprising:

an effective amount of at least one polypeptide selected from the group consisting of gp120, gp41, a variant of gp120, and a variant of gp41; and

a pharmaceutically suitable excipient.

2. The pharmaceutical composition of claim 1 wherein the chemoattractant is selected from the group consisting of a C-C chemokine, SDF-1α, and fMLP.

3. The pharmaceutical composition of claim 2 wherein the C-C chemokine is selected from the group consisting of MIP-1α, MIP-1β, RANTES, MCP-1, and MCP-3.

4. The pharmaceutical composition of claim 1 wherein the CD4+ cell is selected from the group consisting of monocytes and T lymphocytes.

5. The pharmaceutical composition of claim 1 wherein the variant of gp120 and gp41 comprise CD4-binding fragments of gp120 and gp41, respectively.

6. The pharmaceutical composition of claim 1 wherein the polypeptide comprises a V3 loop of gp120.

7. The pharmaceutical composition of claim 1 wherein the polypeptide comprises a PND domain of gp120.

8. The pharmaceutical composition of claim 1 comprising an effective amount of a polypeptide comprising peptide F.

9. The pharmaceutical composition of claim 1 wherein the composition further comprises an anti-inflammatory substance other than gp120, gp41, and variants thereof.

10. A method of reducing a chemotactic response of a $CD4^+$ cell comprising administering a pharmaceutical composition comprising an effective amount of a CD4-binding compound.

11. The method of claim 10 wherein the CD4-binding compound is selected from the group consisting of gp120, gp41, variants of gp120, variants of gp41, and an anti-CD4 antibody.

12. The method of claim 10 wherein the $CD4^+$ cell is a monocyte or T lymphocyte.

13. The method of claim 10 wherein the pharmaceutical composition comprises an amount of the polypeptide that is effective to treat an inflammatory condition.

14. The method of claim 13, wherein the inflammatory condition is selected from the group consisting of rheumatoid arthritis, glomerulonephritis, multiple sclerosis, myocarditis, a hypersensitivity syndrome, an allergic reaction, and asthma.

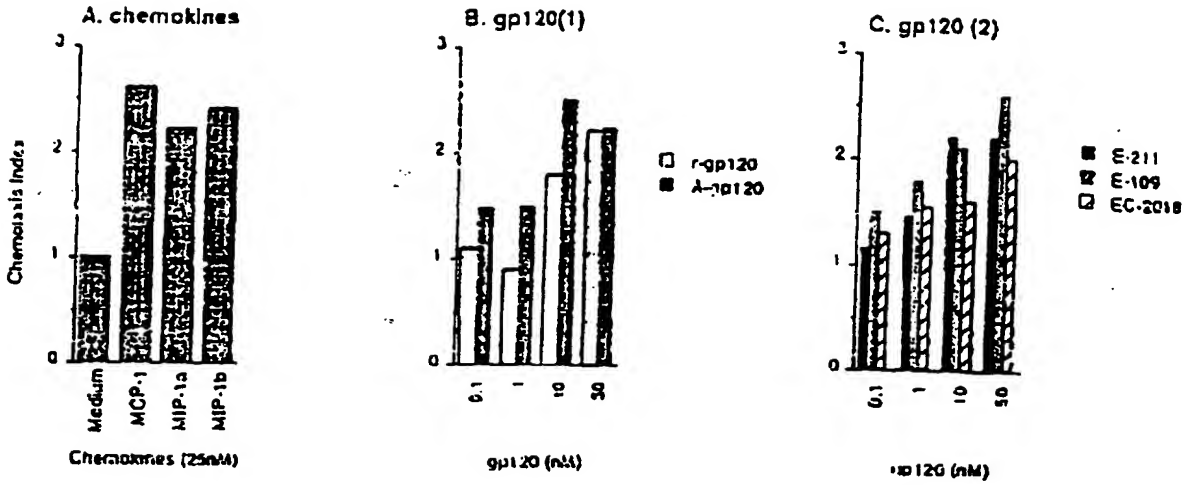

A. chemokines
Chemotaxis Index
Medium
MCP-1
MIP-1a
MIP-1b
Chemokines (25nM)
B. gp120(1)
r-gp120
A-10120
gp120 (nM)
C. gp120 (2)
E-211
E-109
EC-2018

FIGURE 1

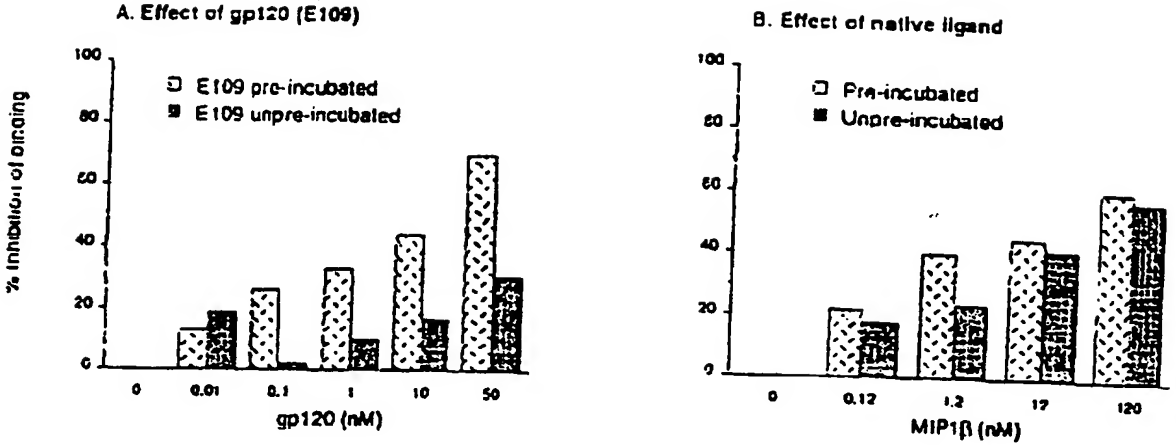

A. Effect of gp120 (E109)
% Inhibition of binding
E109 pre-incubated
E109 unpre-incubated
100
80
60
40
20
0
0
0.01
0.1
1
10
50
gp120 (nM)
B. Effect of native ligand
Pre-incubated
Unpre-incubated
100
80
60
40
20
0
0
0.12
1.2
12
120
MIP1β (nM)

FIGURE 2

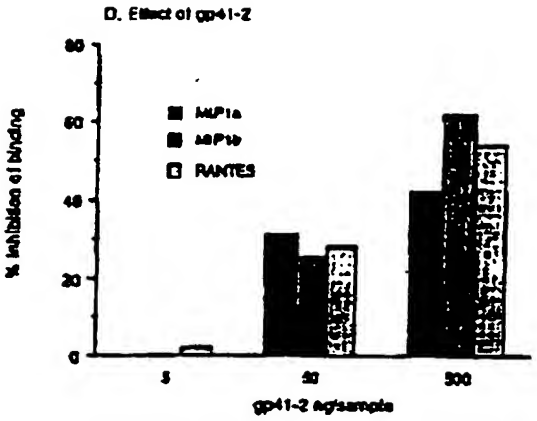

D. Effect of gp41-2
% Inhibition of Binding
MIP1a
MIP1b
RANTES
0
20
40
60
80
5
50
500
gp41-2 ng/sample

FIGURE 3

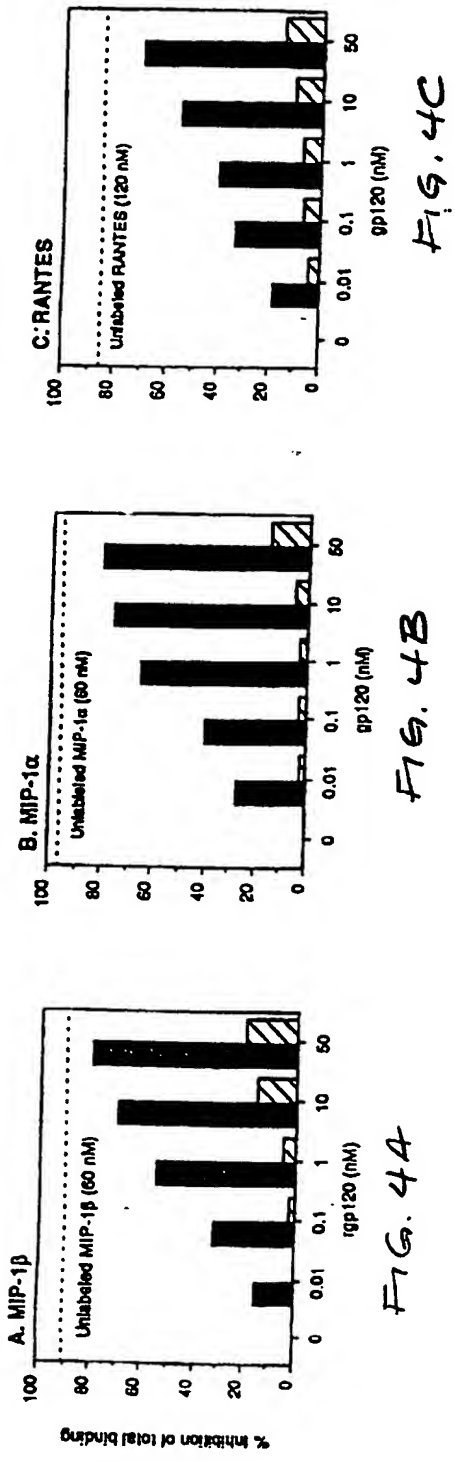


FIG. 4A

FIG. 4B

FIG. 4C

FIG. 4D

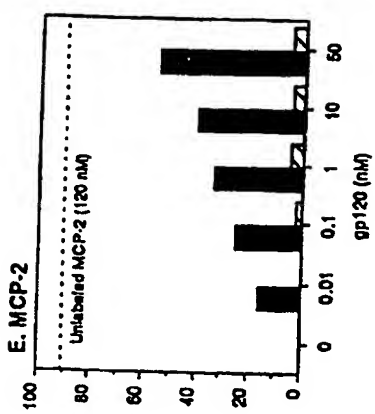


FIG. 4E

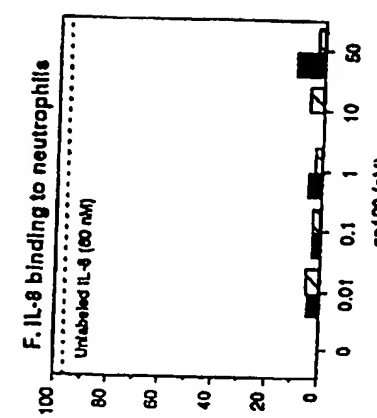


FIG. 4F

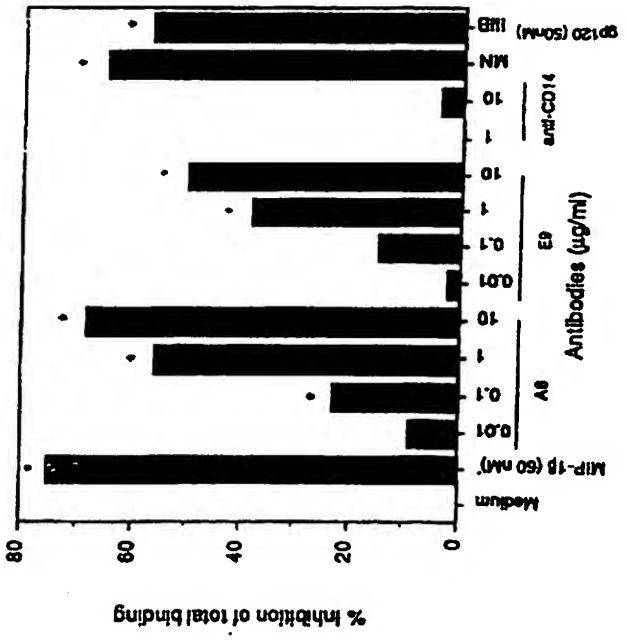

% inhibition of total binding
0
20
40
60
80
medium
MIP-1β (60 nM)
0.01
0.1
1
10
4B
0.01
0.1
1
10
E9
anti-CD14
1
10
MN
IIIB
gp120 (50nM)
Antibodies (μg/ml)

FIG. 5

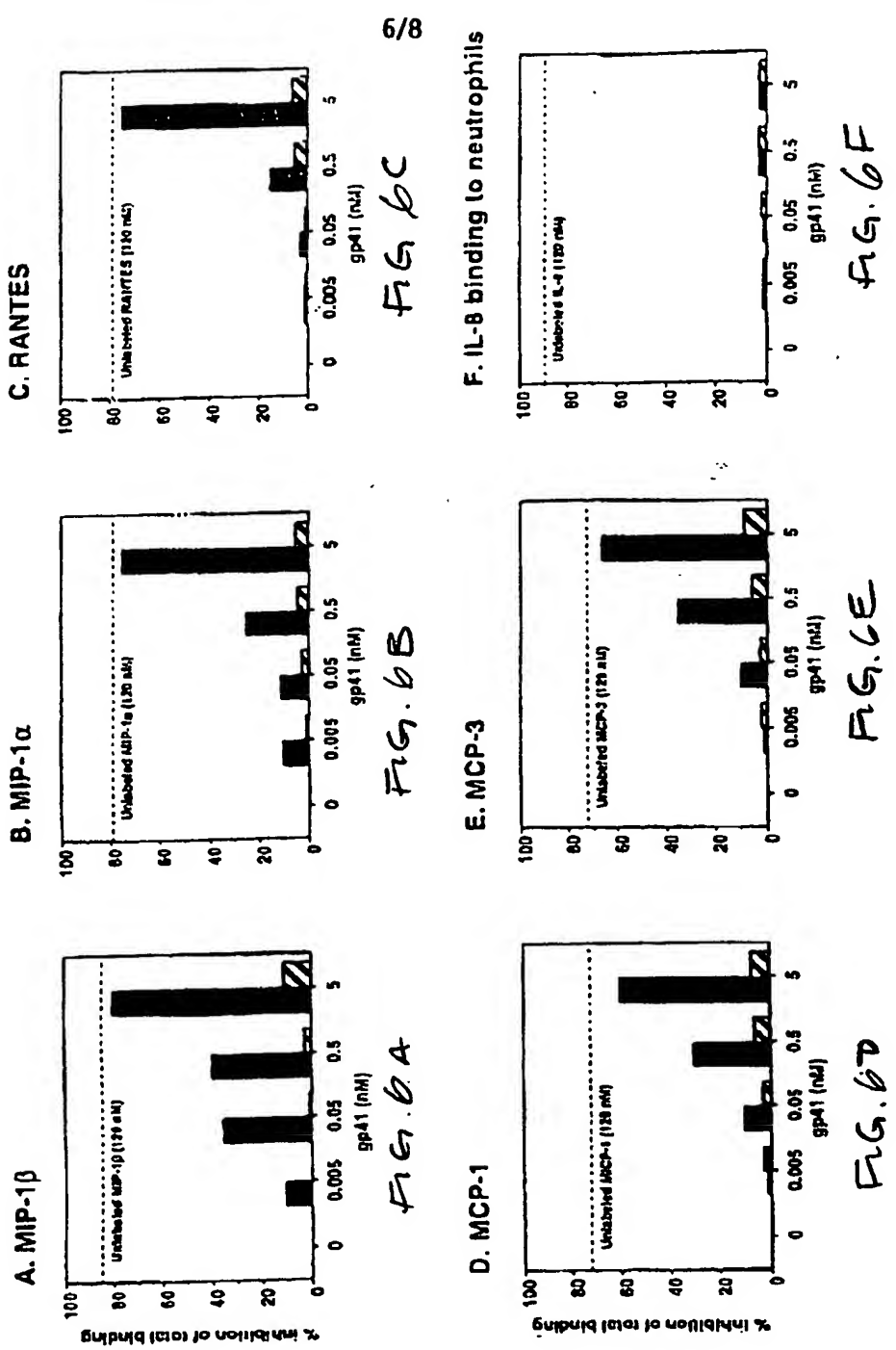


FIG. 6A FIG. 6B FIG. 6C

FIG. 6D FIG. 6E FIG. 6F

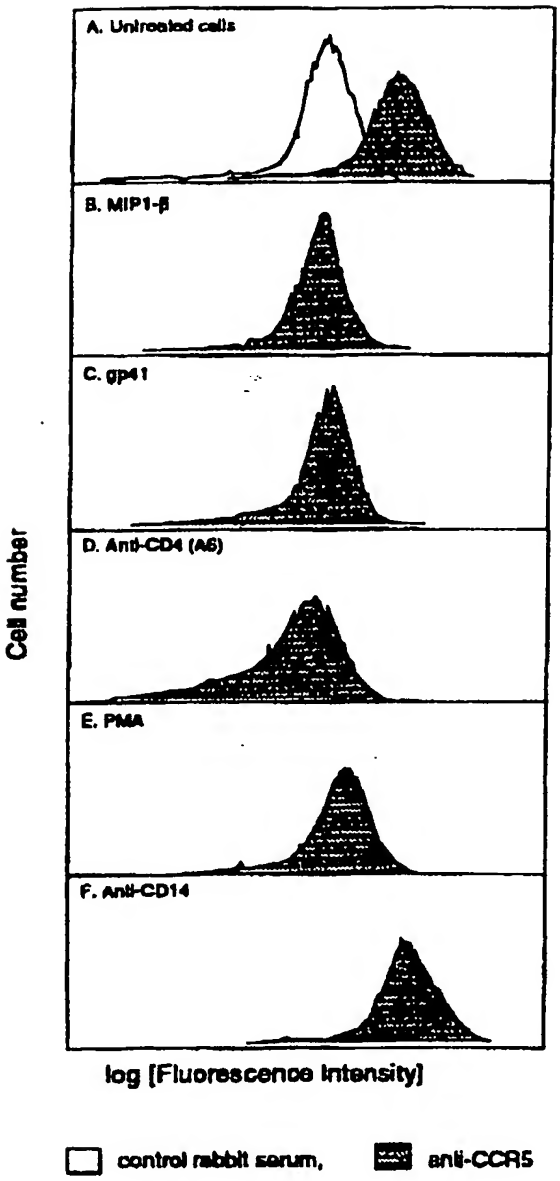
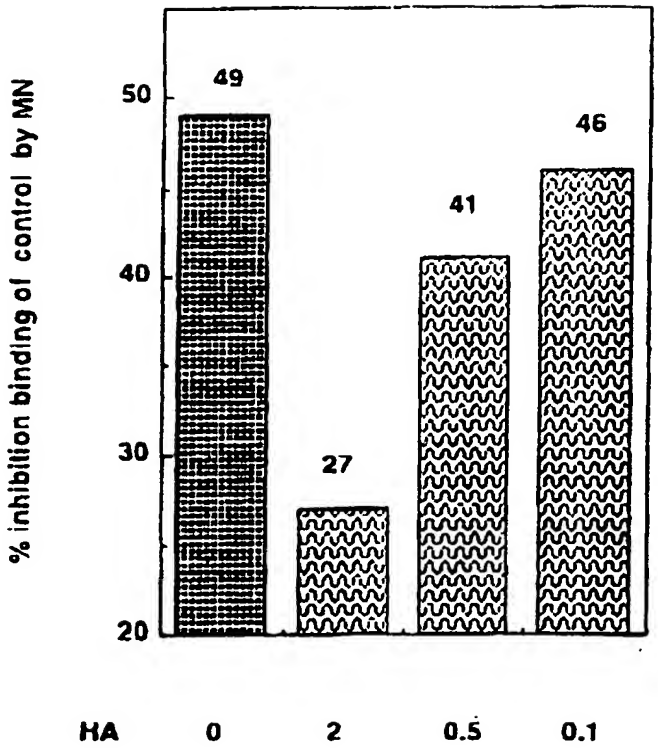

A. Untreated cells
B. MIP1-β
C. gp41
D. Anti-CD4 (A6)
E. PMA
F. Anti-CD14
Cell number
log [Fluorescence Intensity]
control rabbit serum,
anti-CCR5

FIG. 7



The reversible effect of Herbimycin A on inhibition of SDF-1α binding by CEM-SS cells incubated with gp120-MN. 1 x 10^6 CEM cells/ml in 0.5% FBS-RPMI1640 were incubated with herbimycin A at indicated concentration for 18 hrs at 37 °C and then the binding assay was performed. [1 x 10^6 cells/tube/200μl. gp-120-MN: 50 nM, room temperature for 60 min].

FIG. 8

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